Metal Chelation as a Potential Therapy for Alzheimer's Disease

MATH P. CUAJUNGCO,[a] KYLE Y. FAGÉT,[a] XUDONG HUANG,[a] RUDOLPH E. TANZI,[b] AND ASHLEY I. BUSH[a,c]

[a]*Laboratory for Oxidation Biology, Massachusetts General Hospital, and Department of Psychiatry, Harvard Medical School, Boston, Massachusetts 02115, USA*

[b]*Genetics and Aging Unit, Massachusetts General Hospital, Charlestown, Massachusetts 02129, USA*

ABSTRACT: **Alzheimer's disease is a rapidly worsening public health problem. The current lack of effective treatments for Alzheimer's disease makes it imperative to find new pharmacotherapies. At present, the treatment of symptoms includes use of acetylcholinesterase inhibitors, which enhance acetylcholine levels and improve cognitive functioning. Current reports provide evidence that the pathogenesis of Alzheimer's disease is linked to the characteristic neocortical amyloid-β deposition, which may be mediated by abnormal metal interaction with Aβ as well as metal-mediated oxidative stress. In light of these observations, we have considered the development of drugs that target abnormal metal accumulation and its adverse consequences, as well as prevention or reversal of amyloid-β plaque formation. This paper reviews recent observations on the possible etiologic role of Aβ deposition, its redox activity, and its interaction with transition metals that are enriched in the neocortex. We discuss the effects of metal chelators on these processes, list existing drugs with chelating properties, and explore the promise of this approach as a basis for medicinal chemistry in the development of novel Alzheimer's disease therapeutics.**

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by extracellular deposits of amyloid-β protein (Aβ), the main component of neuritic or senile and diffuse plaques.[1] Pathogenic mutations of the APP gene close to or within the Aβ domain are linked to forms of familial AD (FAD).[2] Inheritance of mutations on chromosome 14 (presenilin-1),[3] or chromosome 1 (presenilin-2)[4] produces the more aggressive form of the disease (early-onset age of 25–45 years). Moreover, apolipoprotein-E (apoE) ε4 allele on chromosome 19 has been identified

[c]Address for correspondence: Dr. Ashley Bush, Director, Laboratory for Oxidation Biology, Genetics & Aging Unit MGH, 13th Street Bldg. 149, Charlestown, MA 02129. Tel.: (617) 726-8244; fax: (617) 724-9610.
e-mail: BUSH@helix.mgh.harvard.edu

Annals NY Acad Sci 920: 292-304, 2000.

as a risk factor for late-onset AD.[5] More recently, a genetic deletion of the α_2-macroglobulin (A2M) gene on chromosome 12 was discovered to be another risk factor for AD.[6]

Although the effects of the genetic lesions that cause FAD are to elevate $A\beta_{1-42}$ levels,[7] the mere presence of $A\beta_{1-42}$ cannot initiate amyloid deposition since the peptide is a normal component of healthy CSF.[8] If elevated cortical Aβ concentrations were solely responsible for the initiation of amyloid, it would be difficult to explain why the amyloid deposits are focal (related to synapses and the cerebrovascular lamina media) and not uniform in their distribution. Importantly, overexpression of $A\beta_{1-42}$ from birth, which occurs in genetic forms of AD (FAD and Down's syndrome), does not induce amyloid deposition in childhood.[9] In these cases, Aβ deposition still occurs in an age-dependent, albeit accelerated manner. Also, we have found the total levels of Aβ measured in postmortem brain tissue from AD cases are increased to the same extent in both brain regions that form abundant amyloid (e.g., hippocampus) compared to tissues that do not form amyloid (such as cerebellum).[10] From these observations, it seems unlikely that Aβ overproduction alone initiates Aβ deposition, and thus, it is more likely that there are neurochemical factors, altered as a stochastic consequence of aging, that initiate Aβ deposition in sporadic AD and FAD. The plaque deposits of Aβ appear then to be a morphological variation of Aβ accumulation caused by neurochemical interactions that are specific to the neocortex. The availability of high concentrations of Cu(II) and Zn(II) is a specific feature of neocortical tissue that could explain the condensation of Aβ as plaque. Here, we review current evidence for abnormal metal interactions in AD and discuss the potential therapeutic effect of metal chelators against AD pathology.

CEREBRAL ZINC, COPPER AND IRON LEVELS IN AD

There is an emerging consensus in the literature to indicate that the homeostases of zinc, copper, and iron are significantly altered in the AD brain tissue (reviewed in Refs. 11,12). For example, abnormal levels of zinc, copper, or iron have been found in several subcortical regions such as the hippocampus, amygdala, and olfactory bulb, as well as the neocortex.[11,12] A recent well-controlled study using microparticle-induced X ray emission (PIXE) analysis of the cortical and accessory basal nuclei of the amygdala indicated that zinc, copper, and iron accumulate in the neuropil and plaques of the AD brain where their concentrations are 3–5-fold increased compared to age-matched controls (TABLE 1). In fact, the concentrations of these metal ions, particularly the redox active Cu and Fe (implicated in free radical reactions)[13] are normally concentrated in those regions of the brain most affected by AD pathology. Evidence for abnormal Cu homeostasis in AD includes a 2.2-fold increase in the concentration of CSF Cu,[14] and an accompanying increase in ceruloplasmin in the brain and CSF of AD patients.[15] Similarly, there is an extensive literature describing abnormal levels of Fe and Fe-binding proteins in AD.[16] It has been demonstrated that the Fe found within the amyloid deposits of human brains and in amyloid-bearing APP transgenic mice brains is redox-active.[17]

TABLE 1. Micro-PIXE analysis of metal ion concentrations in Alzheimer's disease plaque and neuropil

	Zinc μg/g (μM)[a]	Copper μg/g (μM)*	Iron μg/g (μM)*
Senile plaque	69 (1055)	25 (393)	53 (940)
AD neuropil	51 (786)	19 (304)	39 (695)
Control neuropil	23 (346)	4 (69)	19 (338)

[a]Adapted from Ref. 18. For purposes of comparison we have converted the published values into molar concentrations assuming a sample density of 1 g/cm^3.

BIOMETALS AND RISK FACTORS FOR ALZHEIMER'S DISEASE

The metal binding proteins, A2M[19] and apoE,[20] are typically found in senile plaques.[21] A2M mediates Aβ degradation via its low density-related lipoprotein (LRP) receptor.[22] A2M binding with Aβ, which is enhanced by the presence of zinc, precludes Aβ fibrillogenesis and reduces its associated neurotoxicity.[23] Meanwhile, of the three apoE isoforms, the ε4 isoform has been found to be the least effective in inhibiting Cu(II)- and Zn(II)-induced precipitation of Aβ,[20] a finding compatible with the apoE ε4 allele being an independent risk factor for AD.[5] It is interesting to note that AD patients carrying the apoE ε4 allele have been found to have elevated serum zinc and copper levels, providing an association between abnormal metal metabolism and apoE risk for AD.[24] Both iron and copper have been shown to enhance the toxicity of Aβ in cultures,[25,26] and copper toxicity is enhanced by nontoxic concentrations of Aβ via Aβ-mediated glutathione depletion.[27]

APP AND Aβ ARE METAL-BINDING PROTEINS

Investigating the role of brain biometals as a potential target for therapeutic remedies in AD stemmed from a series of *in vitro* studies that found that synthetic Aβ and purified APP exhibited several physicochemical interactions with Zn(II), Cu(II), and to a lesser extent, Fe(III), at low micromolar and submicromolar concentrations of the metal ions.[28–34] Hence, disruption in the homeostasis of these metals could possibly contribute to abnormal metal-Aβ interactions in AD.

Specific and saturable binding sites for zinc (APP 181–200; K_a = 750 nM)[28] and copper (APP 135–155; K_d = 10 nM)[35] were identified within the cysteine-rich region on the ectodomain of APP 695. These sites have homology in all known members of the APP superfamily.[31] This indicates that zinc and copper interaction with the protein may play an important, evolutionary conserved role in APP function and metabolism.

While Zn(II) binding to APP is believed to have a structural role, APP can reduce Cu(II) to Cu(I), which results in oxidation of Cys-144 and Cys-155 and a corresponding intramolecular disulfide bridge formation.[36] The resulting APP-Cu(I) complex is prone to redox reactions that result in site-specific APP fragmentation.[37] The Cys-144 residue of APP was determined to be necessary for this chemical reaction.[38]

$A\beta_{1-40}$ specifically and saturably binds zinc, manifesting high-affinity binding (K_d = 107 nM) with a 1:1 (zinc:Aβ) stoichiometry, and low-affinity binding (K_d = 5.2 μM) with a 2:1 stoichiometry.[29] This binding is histidine-mediated, since it is abolished by acidic pH, and by chemical blocking of the histidine residues.[34] More importantly, His-13 is believed to be an important residue in zinc-mediated Aβ assembly.[39] The zinc binding site was mapped to a stretch of contiguous residues between positions 6–28 of the Aβ sequence. Occupation of the zinc binding site, which straddles the lysine 16 position of α-secretase site, inhibits α-secretase type (tryptic) cleavage and so may influence the generation of Aβ from APP, and may increase the biological half-life of Aβ by protecting the peptide from proteolytic attack.[30] Zinc concentrations above 300 nM rapidly precipitate synthetic human $A\beta_{1-40}$.[29,32,33] Interestingly, zinc may also preserve the α-helical conformation of $A\beta_{1-40}$, which may explain why precipitation of Aβ by Zn(II) is reversible, and that Zn(II)-assembled Aβ can be resolubilized by chelation.[33]

Aβ is also precipitated by Cu(II) in a reaction that is potentiated by mildly acidic (pH 6.6) conditions. The stoichiometry of Cu:Aβ increases from zero when Aβ is soluble to 1.0–2.5 when Aβ is aggregated by Cu(II).[34,40] $A\beta_{1-40}$ has higher-affinity (log K_{app} 10) and lower-affinity (log K_{app} 7.0) binding sites for Cu, but the affinity of Cu for $A\beta_{1-42}$ is greater for both sites (log K_{app} 17.3 and log K_{app} 8.0, respectively).[40] The high-affinity Cu(II) binding site on $A\beta_{1-42}$ is of such high affinity that it is very likely to be occupied *in vivo*. However, under mildly acidic conditions (pH 6.8), the affinity of $A\beta_{1-40}$ and $A\beta_{1-42}$ for Zn(II), but not Cu(II) decreases at the lower-affinity binding sites.[40]

Unlike Zn(II), both Cu(II) and Fe(III) induce greater Aβ aggregation under mildly acidic conditions (e.g., pH 6.8–7.0).[32,34] However, Aβ binds equimolar amounts of Cu(II) and Zn(II) at pH 7.4, while Cu(II) displaces Zn(II) from Zn(II):Aβ aggregates under acidic conditions (pH 6.6).[40] $A\beta_{1-42}$ markedly aggregates in the presence of trace amounts (<0.1 μM) of Cu(II).

OXIDATIVE MECHANISMS OF Aβ NEUROTOXICITY

Both Aβ deposition in the neocortex, and oxidative stress, are considered closely related to the pathogenesis of AD. The deposition of Aβ in the neocortex of APP transgenic mice overexpressing Aβ is accompanied by some neuropathological features of AD such as neuronal loss,[41] and signs of oxidative damage[42] suggesting that the neurotoxic events of AD are seminally related to Aβ accumulation. Many studies have now confirmed that Aβ is neurotoxic in cell culture[43] and *in vivo*.[44] Therefore, prevention of Aβ deposition could be a therapeutic target in AD.

Focus on the pathogenic relevance of oxidative stress in AD was stimulated by the report that treatment of AD subjects with the antioxidant vitamin E delays de-

cline in independent functioning.[45] Metabolic signs of oxidative stress such as oxygen radical-mediated damage of brain proteins, lipids, and nucleic acids, as well as systemic signs of oxidative stress and the response of antioxidant systems have all been observed in AD.[46,47] The biochemical relationship between Aβ deposition in AD and oxidative stress is complex, and the mechanisms underlying the association between oxidation and amyloid deposition are not well understood.

Synthetic Aβ peptides exert toxicity through mechanisms that involve the generation of cellular hydrogen peroxide (H_2O_2).[48] The observed cytotoxicity is abolished by $O_2^{-\bullet}/H_2O_2$ scavengers.[49] Taken together, the chemical nature of oxidation stress in AD indicates that H_2O_2 levels may be elevated in the AD brain. While Zn(II) is redox-inert, we recently reported that the binding of trace concentrations of redox-active metals Cu(II) and Fe(III) to Aβ engenders the cell-free catalytic production of H_2O_2 from O_2 via metal reduction.[50] The redox activities of Aβ species are greatest for $A\beta_{42}$>$A\beta_{40}$>>rat $A\beta_{40}$, a chemical relationship that corresponds to the participation of the respective peptide in amyloid pathology, as well as the peptide's H_2O_2-mediated toxicity in cell culture.[26] It is interesting to note that the Aβ peptide has a selective vulnerability to Cu-mediated OH• attack that oxidizes the peptide, emulating the chemical changes seen in the Aβ extracted from AD brain (Atwood *et al.* submitted).

METAL COMPLEXING PROPERTIES OF CHELATING AGENTS: AN OVERVIEW

The term chelator originated from the Greek word "chele," which means "crab's claw."[51,52] This term defines the complexes formed by a ligand (a molecule with at least two donor groups or coordination number) with their substrates (ions) such that a "ring" system is established. The process of creating such a ring structure is well correlated with the formation of a more stable complex.[52] Denticity (from the word "dens," meaning tooth) is used to describe the number of available donor groups of a chelating agent to bind metal ions.[52] For example, bidentate refers to two donor groups, tridentate to three, quinquidentate to five, and so forth. Some chelators are able to form multidentate complexes, while others can only attain a monodentate or bidentate chelate rings. For multidentate ligands, the dissociation constant (K_d; a constant that reflects the intrinsic strength of metal-ligand binding) can vary markedly for different species.[51] Some chelators have the ability to directly permeate cell membranes prior to or upon binding metal ions (e.g., see Ref. 53). Other chelators become membrane permeable after esterification, or by acquiring a nonpolar state following metal complexation.[51] In addition, some chelators are ionophores since their chelating sites have limited flexibility and thus would prefer cations that fit easily into their molecular structure.[52] Ionophores may selectively enhance the permeability of metal ions in lipid membranes of cells as in the case of calcium ionophore A23187 (calcimycin), which facilitates entry of calcium ions into cells.[54] Similarly, pyrithione is a zinc chelator that neutralizes zinc neurotoxicity, but also has an ionophoric property.[53] Hence chelators may act to either deprive biological systems of metal ions, or may have the opposite effect of promoting metal uptake into cells.

EFFECTS OF METAL CHELATORS ON Aβ PATHOPHYSIOLOGY

Inhibition of the Redox Activity and Aggregation of Aβ

FAD-linked mutations of APP, presenilin-1 and presenilin-2, increase both Aβ amyloid burden and $A\beta_{1-42}$ production, underscoring the role that the longer Aβ species plays in AD pathogenesis.[7] Hence, much effort has focused on the mechanisms of $A\beta_{1-42}$-mediated fibrillogenesis. A landmark *in vitro* study in this area was that of Jarrett *et al.*[55] who reported that $A\beta_{1-40}$, which is kinetically stable at 20 μM in solution for nine days, is destabilized over that interval by "seeding" with 2 μM $A\beta_{1-42}$ fibrils. This work has been considerably augmented and has lead to a theory of nucleation-dependence or "seeding" of amyloidogenic peptides in several neurodegenerative diseases. We extended the work of Jarrett *et al.*[55] and found that addition of Zn(II), Cu(II), and Fe(III) enhanced $A\beta_{1-42}$-initiated seeding of $A\beta_{1-40}$ (Huang *et al.* submitted). However, we measured the concentrations of these metal ions that contaminate the incubation buffer and found levels to be 0.1–0.5 μM, great enough to precipitate Aβ. Therefore, we tested the effects of chelators upon this classic nucleation reaction, and found that polyamincarboxylic acid compounds like diethylene tetraamine pentaacetic acid (DTPA), and cyclohexane diamine tetraacetic acid (CDTA), both high-affinity Cu(II) and Zn(II) chelators, abolished the seeding reaction. Furthermore, NMR spectroscopy proved that these compounds do not interact directly with the peptide. These findings indicate that metal ions are essential for the initiation of nucleation-dependent fibrillogenesis.

We have shown that Aβ reduces Cu and Fe, and that Aβ can generate H_2O_2 through a metal-dependent reaction.[50] We thus tested the possibility that metal chelators could interfere with the redox activity of Aβ. We observed that BP and DTPA abolished (at a 200-fold molar excess) the H_2O_2 generated by Aβ (10 μM) interacting with Fe(III)-citrate (1 μM).[50] We repeated this experiment using a 200-molar excess of desferrioxamine (DFO, a high-affinity Fe[III] chelator), and found that DFO was ineffective in preventing H_2O_2 formation unlike BP and DTPA.[56] These results suggest that the ability of a compound to inhibit Aβ:metal-mediated redox activity is not simply a product of its affinity, but that other factors such as stereochemistry of the metal binding site, play important roles.

Resolubization of Aβ Plaques in Vitro *and in Postmortem Human and APP Transgenic Mouse Brain Tissue*

We have shown *in vitro* that zinc-induced[33] or copper-induced[34] precipitation of Aβ peptide is a chelation-reversible event. Interestingly, zinc-precipitated Aβ is denser and less easily resolubilized than copper-induced precipitates.[20] Indeed, Aβ is resolubilized and extracted from postmortem AD and non-AD control brains using metal chelators.[57] High-affinity Cu/Fe/Zn chelators such as N,N,N′,N′-tetrakis-(2-pyridylmethyl)-ethylenediamine (TPEN) and bathocuproine disulfonic acid (BC), markedly enhanced the resolubilization of Aβ deposits from postmortem AD and non-AD brain samples.[57] The observed increase in extractable Aβ correlated with significant depletion in zinc (30%) and to a lesser extent, copper, in each of the AD cases examined ($n = 10$) when compared with PBS-alone treated tissue.[57] The ability

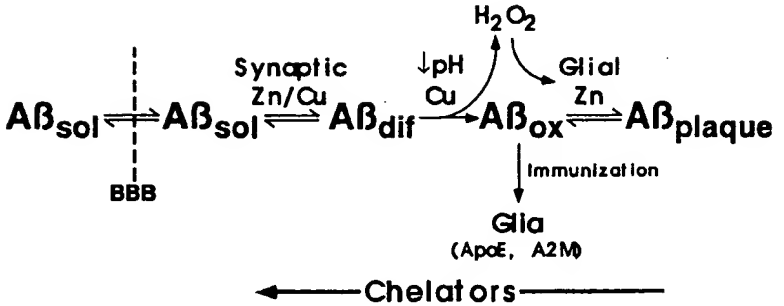

FIGURE 1. A model for the role of metal/Aβ interaction in AD. Aβ is a normal soluble constituent of biological fluids including plasma and brain interstitial fluids ($A\beta_{sol}$). During epochs of metabolic stress (e.g., head injury, hypoxia, etc.), the uptake of zinc and copper via energy-dependent uptake mechanisms after they are released into the synapse is inhibited. This raises the concentration of zinc and copper in the synaptic vicinity, precipitating Aβ at that site as diffuse amyloid deposits on histology ($A\beta_{dif}$). These deposits would ordinarily resolve as the metabolic stress diminishes and the interstitial zinc and copper concentrations decrease to normal again. If on the other hand, the concentration of Cu(II) remains high then Cu(II) may displace Zn(II) from Aβ (especially under acidotic conditions)[34] abnormally generating H_2O_2 and oxidizing Aβ (carbonyl modification). Soluble oxidized forms of Aβ ($A\beta_{ox}$) are protease resistant,[64] and therefore abnormally long-lived in the cortical interstitium where they drive up the H_2O_2 levels even more, taxing the cellular scavenging mechanisms (e.g., glutathione), and contributing to neurotoxicity.[25–27] The elevated H_2O_2, being freely permeable, crosses the membrane of the neighboring glia and reacts with metallothionein, causing the liberation of the metallothionein-held pool of Zn(II).[11,65] The Zn(II) liberated into the interstitium by the glia precipitates the oxidized forms of Aβ into plaque deposits that, as a result, have very high (~1 mM) levels of Zn(II)[18,66,67] but no longer produce H_2O_2 so that plaque deposits become sites of decreased oxidative damage to neighboring tissue.

The plaque contains oxidized protease-resistant Aβ that is cemented together by Zn(II), which induces even further resistance to proteolysis,[30] so generating a deposit that defies clearance until disaggregated by a chelator.[57,58] Treatment with a compound that complexes both Zn(II) and Cu(II) promotes the dissolution of Aβ plaque and diffuse deposits[57,58] while simultaneously shutting down H_2O_2 production,[26,50] and facilitating the clearance of damaged forms of Aβ by the glia. Damaged/oxidized forms of Aβ may be antigenically foreign, and cleared by activated glia, perhaps explaining how immunization of transgenic animals with synthetic Aβ may work to clear Aβ by promoting glial recognition of oxidized Aβ forms.[68]

Synaptic zinc is more likely than copper to be responsible for the initial precipitation of Aβ as diffuse deposits, because: (a) Zn(II) induces the precipitation of Aβ far more extensively at pH 7.4 *in vitro* than does Cu(II);[30,34] (b) the concentration of synaptic Zn(II) reached during neurotransmission is 10–20-fold higher than that reached by Cu(II);[69,70] and (c) the distribution of vesicular (synaptically released) Zn(II) in the brain corresponds to the sites of the brain that are most prone to amyloid pathology—the cerebral cortex, and not the subcortical gray matter or the cerebellum.[69]

of a chelator to extract Aβ depended upon the presence of Mg(II) and Ca(II), hence the chelating compound needed must be more selective for Zn(II) and Cu(II), than Ca(II) and Mg(II). Higher concentrations of Cu/Zn chelator caused a paradoxical decrease in the amount of Aβ released, because the sequestration of Ca/Mg from the sample became substantial. This work was further extended on APP transgenic

"Hsiao" mouse brain samples using either triethylenetetraamine (TETA; a high-affinity Cu[II] chelator) or bicinchoninic acid (BCA; a Cu[I]-selective chelator) giving similar results to that of the human study.[58]

The Chelating Class of Molecule as a Pharmacological Possibility in AD

Presently, the only AD therapeutic agents approved by the FDA are acetylcholine esterase (AChE) inhibitors (e.g., donepezil), which enhance cholinergic neurotransmission by hindering the breakdown of acetylcholine. This approach, while providing modest clinical gains, is not believed to retard the progression of the underlying disease. Hence, there is a need to find a suitable drug that attacks the disease at its pathogenesis.

We have reviewed findings which suggest that metal complexing agents may have therapeutic benefits in AD. In the last few years, chelation therapy has become increasingly promoted as a therapy for AD, both by individual medical practitioners and by lay groups and even at internet web sites. However, the growing practice of intravenous infusions of EDTA is based upon largely unscientific interpretations of the neurochemical problems in AD, and can lead to systemic metal ion depletion.

One previous clinical trial of the chelator compound DFO was reported to significantly arrest the progression of the disease.[59] Although the DFO trial was thought to target Al(III), it is possible that the beneficial effect of the treatment was due to chelation of Fe(III), Cu(II), and Zn(II). Indeed, the authors reported verbally (International Conference on Alzheimer's Disease, Padua, 1992) that postmortem metal analysis on brain tissue of study-subjects indicated that although aluminum levels were lower than placebo controls, zinc and iron levels were also decreased in the brains of DFO-treated subjects. This is because, like all chelators, DFO has only a relative selectivity for aluminum, and will also complex with zinc, copper, and iron.[60] Although the results of Crapper-McLachlan and colleagues[59] have not yet been reproduced, further consideration of the removal of zinc, copper, and iron from brain Aβ collections as a therapeutic maneuver seems warranted (FIG. 1). DFO is a charged molecule that does not easily penetrate the blood-brain barrier and is easily degraded after it is administered.[52] Further clinical research into the effects of DFO may have been met with diminished enthusiasm, since the administration of DFO is associated with discouraging difficulties including the nonspecific problems of systemic metal ion depletion (e.g., anemia), and the problem of administration of a twice-daily, painful intramuscular injection.

We propose that the metal binding sites on Aβ may provide an appropriate drug target for rational drug design. Although the 3-D structure of Aβ is unknown, our data indicate that the redox active metal binding site on the protein is subject to steric principles, and therefore small compounds with great specificity for this site may be developed. The principles of pharmacotherapeutic molecule complexing a metal-binding site on a protein target is actually well developed in pharmacology. Several well-known antibiotic, anticonvulsive, antitumor, and antiinflammatory drugs (TABLE 2) exert their pharmacological action by interacting with the Cu-, Zn-, or Fe-active site of their target protein. Disulfiram, for example, blocks enzyme activity by chelating the zinc-catalytic site of alcohol dehydrogenase.[61] Nonsteroidal antiinflammatory drugs (NSAIDs) such as aspirin, diflunisal, ibuprofen, naproxen sodium, indomethacin, *d*-penicillamine, etc., block the heme-iron catalytic site on

TABLE 2. A list of drugs that possess chelating properties

Usage	Drug name	Metal Chelate	Reference
Antiinflammatory; Aanalgesic	Aspirin, indomethacin, *d*-penicillamine, ibuprofen	copper, iron, zinc	62,71–73
Antibiotic; antitumor; sedative	Bleomycin, ethambutol, thalidomide	copper, iron, zinc	74-76
Antioxidant; dietary supplement	α-lipoic acid	zinc, copper, manganese	77
Anticonvulsant	Valproate sodium, phenytoin	copper, selenium, zinc	78,79
Alcohol abuse	Tetraethyl thiuram disulfide or diethyl dithiocarbamate (disulfiram/ antabuse)	copper, zinc	53

cyclooxygenase/arachidonic acid pathway.[62] Intriguingly, the use of these drugs has also been reported to reduce the epidemiological risk for AD,[63] but their therapeutic value is still uncertain (see also Progress Report on Alzheimer's Disease, NIA/NIH publication).

CONCLUDING REMARKS

Our current findings indicate that an ideal therapeutic drug to dissolve Aβ amyloid would involve a compound that is relatively selective for Cu(I), Zn(II), and possibly Fe(III), but does not sequester Mg(II) or Ca(II), and that coordinates metal ions in the cerebral amyloid mass but not systemically. Charged species cannot diffuse through biological membranes and thus are confined to the tissue compartment where they were administered. Electrically neutral and nonpolar molecules are ideal chelators, since they are best absorbed across the gastrointestinal tract and achieve a broad distribution throughout various tissues. Finally, tissue and target selectivity of the chelator-drug is essential in order to prevent other biologically important metal ions from becoming systemically depleted during therapy.

REFERENCES

1. GLENNER, G.G. & C. WONG. 1984. Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. Biochem. Biophys. Res. Commun. **120:** 885–890.
2. CHARTIER-HARLIN, M.C., F. CRAWFORD & H. HOULDEN. 1991. Early-onset Alzheimer's disease caused by mutations at codon 717 of the β-amyloid precursor protein gene. Nature **353:** 844–846.
3. SHERRINGTON, R., E.I. ROGAEV, Y. LIANG *et al.* 1995. Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. Nature **375:** 755–760.

4. LEVY-LAHAD, E., E.M. WIJSMAN, E. NEMENS *et al.* 1995. A familial Alzheimer's disease locus on chromosome 1. Science **269:** 970–973.
5. SAUNDERS, A.M., W.J. STRITTMATTER, D. SCHMECHEL *et al.* 1993. Association of apolipoprotein E allele ε4 with late-onset familial and sporadic Alzheimer's disease. Neurology **43:** 1467–1472.
6. BLACKER, D., M.A. WILCOX, N.M. LAIRD *et al.* 1998. Alpha-2 macroglobulin is genetically associated with Alzheimer's disease. Nat. Genet. **19:** 357–360.
7. SCHEUNER, D., C. ECKMAN, M. JENSEN *et al.* 1996. Secreted amyloid β-protein similar to that in the senile plaques of Alzheimer's disease is increased *in vivo* by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. Nat. Med. **2:** 864–870.
8. TAMAOKA, A., N. SAWAMURA, T. FUKUSHIMA *et al.* 1997. Amyloid beta protein 42(43) in cerebrospinal fluid of patients with Alzheimer's disease. J. Neurol. Sci. **148:** 41–45.
9. LEMERE, C.A., J.K. BLUSZTAJN, H. YAMAGUCHI *et al.* 1996. Sequence of deposition of heterogeneous amyloid beta-peptides and apoE in Down syndrome: implications for initial events in amyloid plaque formation. Neurobiol. Dis. **3:** 16–32.
10. MCLEAN, C., R.A. CHERNY, F. FRASER *et al.* 1999. Soluble pool of Aβ amyloid as a determinant of severity of neurodegeneration in Alzheimer's disease. Ann. Neurol. **46:** 860–866.
11. CUAJUNGCO, M.P. & G.J. LEES. 1997. Zinc metabolism in the brain: relevance to human neurodegenerative disorders. Neurobiol. Dis. **4:** 137–169.
12. ATWOOD, C.S., X. HUANG, R.D. MOIR *et al.* 1999. Role of free radicals and metal ions in the pathogenesis of Alzheimer's disease. Metal Ions Biol. Syst. **36:** 309–364.
13. HALLIWELL, B. & J.M.C. GUTTERIDGE. 1984. Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem. J. **219:** 1–14.
14. BASUN, H., L.G. FORSSELL, L. WETTERBERG & B. WINBLAD. 1991. Metals and trace elements in plasma and cerebrospinal fluid in normal aging and Alzheimer's disease. J Neural. Transm. Park. Dis. Dement. Sect. **3:** 231–258.
15. LOEFFLER, D.A., P.A. LEWITT, P.L. JUNEAU *et al.* 1996. Increased regional brain concentrations of ceruloplasmin in neurodegenerative disorders. Brain Res. **738:** 265–274.
16. ROBINSON, S.R., D.F. NOONE, J. KRIL & G.M. HALLIDAY. 1995. Most amyloid plaques contain ferritin-rich cells. Alzheimer's Res. **1:** 191–196.
17. SMITH, M.A., P.L.R. HARRIS, L.M. SAYRE & G. PERRY. 1997. Iron accumulation in Alzheimer's disease is a source of redox-generated free radicals. Proc. Natl. Acad. Sci. USA **94:** 9866–9868.
18. LOVELL, M.A., J.D. ROBERTSON, W.J. TEESDALE *et al.* 1998. Copper, iron and zinc in Alzheimer's disease senile plaques. J. Neurol. Sci. **158:** 47–52.
19. MIYATA, M. & J.D. SMITH. 1996. Apolipoprotein E allele-specific antioxidant activity and effects on cytotoxicity by oxidative insults and beta-amyloid peptides. Nat. Genet. **14:** 55–61.
20. MOIR, R.D., C.S. ATWOOD, D.M. ROMANO *et al.* 1999. Differential effects of apolipoprotein E isoforms on metal-induced aggregation of Aβ using physiological concentrations. Biochemistry **38:** 4595–4603.
21. REBECK, G.W., S.D. HARR, D.K. STRICKLAND & B.T. HYMAN. 1995. Multiple, diverse senile plaque-associated proteins are ligands of an apolipoprotein E receptor, the α_2-macroglobulin receptor/low-density lipoprotein receptor-related protein. Ann. Neurol. **37:** 211–217.
22. QIU, W.Q., W. BORTH, Z. YE *et al.* 1996. Degradation of amyloid β-protein by a serine protease-α_2-macroglobulin complex. J. Biol. Chem. **271:** 8443–8451.
23. DU, Y., K.R. BALES, R.C. DODEL *et al.* 1998. α_2-Macroglobulin attenuates β-amyloid peptide 1–40 fibril formation and associated neurotoxicity in cultured fetal rat cortical neurons. J. Neurochem. **70:** 1182–1188.
24. GONZÁLEZ, C., T. MARTÍN, J. CACHO *et al.* 1999. Serum zinc, copper, insulin and lipids in Alzheimer's disease epsilon 4 apolipoprotein E allele carriers. Eur. J. Clin. Invest. **29:** 637–642.
25. SCHUBERT, D. & M. CHEVION. 1995. The role of iron in beta amyloid toxicity. Biochem. Biophys. Res. Commun. **216:** 702–707.

26. HUANG, X., M.P. CUAJUNGCO, C.S. ATWOOD *et al.* 1999. Cu(II) potentiation of Alzheimer's Aβ neurotoxicity: correlation with cell-free hydrogen peroxide production and metal reduction. J. Biol. Chem. **274:** 37111–37116.
27. WHITE, A.R., A.I. BUSH, K. BEYREUTHER *et al.* 1999. Exacerbation of copper toxicity in primary neuronal cultures depleted of cellular glutathione. J. Neurochem. **72:** 2092–2098.
28. BUSH, A.I., G. MULTHAUP, R.D. MOIR *et al.* 1993. A novel zinc(II) binding site modulates the function of the βA4 amyloid protein precursor of Alzheimer's disease. J. Biol. Chem. **268:** 16109–16112.
29. BUSH, A.I., W.H. PETTINGELL, G. MULTHAUP *et al.* 1994. Rapid induction of Alzheimer Aβ amyloid formation by zinc. Science **265:** 1464–1467.
30. BUSH, A.I., W.H. PETTINGELL, M.D. PARADIS & R.E. TANZI. 1994. Modulation of Aβ adhesiveness and secretase site cleavage by zinc. J. Biol. Chem. **269:** 12152–12158.
31. BUSH, A.I., W.H. PETTINGELL, M.D. PARADIS *et al.* 1994. The amyloid β-protein precursor and its mammalian homologues: evidence for a zinc-modulated heparin-binding superfamily. J. Biol. Chem. **269:** 26618–26621.
32. BUSH, A.I., R.D. MOIR, K.M. ROSENKRANZ & R.E. TANZI. 1995. Zinc and Alzheimer's disease. Science **268:** 1921–1923.
33. HUANG, X, C.S. ATWOOD, R.D. MOIR *et al.* 1997. Zinc-induced Alzheimer's $A\beta_{1-40}$ aggregation is mediated by conformational factors. J. Biol. Chem. **272:** 26464–26470.
34. ATWOOD, C.S., R.D. MOIR, X. HUANG *et al.* 1998. Dramatic aggregation of Alzheimer Aβ by Cu(II) is induced by conditions representing physiological acidosis. J. Biol. Chem. **273:** 12817–12826.
35. HESSE, L., D. BEHER, C.L. MASTERS & G. MULTHAUP. 1994. The beta A4 amyloid precursor protein binding to copper. FEBS Lett. **349:** 109–116.
36. MULTHAUP, G., A. SCHLICKSUPP, L. HESSE *et al.* 1996. The amyloid precursor protein of Alzheimer's disease in the reduction of copper(II) to copper(I). Science **271:** 1406–1409.
37. MULTHAUP, G., T. RUPPERT, A. SCHLICKSUPP *et al.* 1998. Copper-binding amyloid precursor protein undergoes a site-specific fragmentation in the reduction of hydrogen peroxide. Biochemistry **37:** 7224–7230.
38. RUIZ, F.H., M. GONZÁLEZ, M. BODINI 1999. Cysteine 144 is a key residue in the copper reduction by the beta-amyloid precursor protein. J. Neurochem. **73:** 1288–1292.
39. LIU, S.T., G. HOWLETT & C.J. BARROW. 1999. Histidine-13 is a crucial residue in the zinc ion-induced aggregation of the A beta peptide of Alzheimer's disease. Biochemistry **38:** 9373–9378.
40. ATWOOD, C.S., R.C. SCARPA, X. HUANG *et al.* 2000. Characterization of copper interactions with Alzheimer Aβ peptides: identification of an attomolar affinity copper binding site on $A\beta_{1-42}$. J. Neurochem. **75:** 1219–1233.
41. STURCHLER-PIERRAT, C., D. ABRAMOWSKI, M. DUKE *et al.* 1997. Two amyloid precursor protein transgenic mouse models with Alzheimer disease-like pathology. Proc. Natl. Acad. Sci. USA **94:** 13287–13292.
42. SMITH, M.A., K. HIRAI, K. HSIAO *et al.* 1998. Amyloid-beta deposition in Alzheimer transgenic mice is associated with oxidative stress. J. Neurochem. **70:** 2212–2215.
43. YANKNER, B.A., L.K. DUFFY & D.A. KIRSCHNER. 1990. Neurotrophic and neurotoxic effects of amyloid β protein: reversal by tachykinin neuropeptides. Science **250:** 279–282.
44. EMRE, M., C. GEULA, B.J. RANSIL & M.M. MESULAM. 1992. The acute neurotoxicity and effects upon cholinergic axons of intracerebrally injected beta-amyloid in the rat brain. Neurobiol. Aging **13:** 553–559.
45. SANO, M., C. ERNESTO, R.G. THOMAS *et al.* 1997. A controlled trial of selegiline, alpha-tocopherol, or both as treatment for Alzheimer's disease. The Alzheimer's Disease Cooperative Study. N. Engl. J. Med. **336:** 1216–1222.
46. MECOCCI, P., U. MACGARVEY & M.F. BEAL. 1994. Oxidative damage to mitochondrial DNA is increased in Alzheimer's disease. Ann. Neurol. **36:** 747–751.
47. SMITH, M.A., P.L. RICHEY, S. TANEDA *et al.* 1994. Advanced Maillard reaction end products, free radicals, and protein oxidation in Alzheimer's disease. Ann. N.Y. Acad. Sci. **738:** 447–454.

48. BEHL, C., J.B. DAVIS, R. LESLEY & D. SCHUBERT. 1994. Hydrogen peroxide mediates amyloid β protein toxicity. Cell **77:** 817–827.
49. BRUCE, A.J., B. MALFROY & M. BAUDRY. 1996. β-Amyloid toxicity in organotypic hippocampal cultures: protection by Euk-8, a synthetic catalytic free radical scavenger. Proc Natl. Acad. Sci. USA **93:** 2312–2316.
50. HUANG, X., C.S. ATWOOD, M.A. HARTSHORN *et al.* 1999. The Aβ peptide of Alzheimer's disease directly produces hydrogen peroxide through metal ion reduction. Biochemistry **38:** 7609–76016.
51. MELLOR, D.P. 1964. Historical background and fundamental concepts. *In* Chelating Agents and Metal Chelates. F.P. Dwyer & D.P. Mellor, Eds.: 1–50. Academic Press. New York.
52. MAY, P.M. & R.A. BULMAN. 1983. The present status of chelating agents in medicine. Prog. Med. Chem. **20:** 225–336.
53. CUAJUNGCO, M.P. & G.J. LEES. 1998. Diverse effects of metal chelating agents on the neuronal cytotoxicity of zinc in the hippocampus. Brain Res. **799:** 97–107.
54. BLAU, L., R.B. STERN & R. BITTMAN. 1984. The stoichiometry of A23187- and X537A-mediated calcium ion transport across lipid bilayers. Biochim. Biophys. Acta **778:** 219–223.
55. JARRETT, J.T., E.P. BERGER & P.T LANSBURY, JR. 1993. The carboxy terminus of the β amyloid protein is critical for the seeding of amyloid formation: implications for the pathogenesis of Alzheimer's disease. Biochemistry **32:** 4693–4697.
56. BUSH, A.I., X. HUANG & D.P. FAIRLIE. 1999. The possible origin of free radicals from amyloid β peptides in Alzheimer's disease. Neurobiol. Aging **268:** 335–337.
57. CHERNY, R.A., J.T. LEGG, C.A. MCLEAN, *et al.* 1999. Aqueous dissolution of Alzheimer's disease Aβ amyloid deposits by biometal depletion. J. Biol. Chem. **274:** 23223–23228.
58. GRAY, D.N., R.A. CHERNY, C.L. MASTERS *et al.* 1998. Resolubilization of Alzheimer and APP transgenic beta amyloid plaque by copper chelators [abstract]. Soc. Neurosci. Abstr. **24:** 722.
59. CRAPPER-MCLACHLAN, D.R., A.J. DALTON, T.P.A. KRUCK *et al.* 1991. Intramuscular desferrioxamine in patients with Alzheimer's disease. Lancet **337:** 1304–1308.
60. HIDER, R.C. & A.D. HALL. 1991. Clinically useful chelators of tripositive elements. Prog. Med. Chem. **28:** 41–173.
61. LANGELAND, B.T. & J.S. MCKINLEY-MCKEE. 1996. The effects of disulfiram on equine hepatic alcohol dehydrogenase and its efficiency against alcoholism: vinegar effect. Alcohol Alcohol. **31:** 75–80.
62. RAO, G.H. & J.G. WHITE. 1985. Comparative pharmacology of cyclooxygenase inhibitors on platelet function. Prostaglandins Leukotrienes Med. **18:** 119–131.
63. MCGEER, P.L., M. SCHULZER & E.G. MCGEER. 1996. Arthritis and anti-inflammatory agents as possible protective factors for Alzheimer's disease: a review of 17 epidemiologic studies. Neurology **47:** 425–432.
64. STADTMAN, E.R. 1992. Protein oxidation and aging. Science **257:** 1220–1224.
65. CUAJUNGCO, M.P. & G.J. LEES. 1997. Zinc and Alzheimer's disease: is there a direct link? Brain Res. Rev. **23:** 219–236.
66. LEE, J.-Y., I. MOOK-JUNG & J.-Y. KOH. 1999. Histochemically reactive zinc in plaques of the Swedish mutant beta-amyloid precursor protein transgenic mice. J. Neurosci. **19**(RC10): 1–5.
67. SUH, S.W., K.B. JENSEN, M.S. JENSEN *et al.* 1999. Histological evidence implicating zinc in Alzheimer's disease. Brain Res. **852:** 274–278.
68. SCHENK, D., R. BARBOUR, W. DUNN, *et al.* 1999. Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. Nature **400:** 173–177.
69. FREDERICKSON, C.J. 1989. Neurobiology of zinc and zinc-containing neurons. Int. Rev. Neurobiol. **31:** 145–328.
70. HARTTER, D.E. & A. BARNEA. 1988. Brain tissue accumulates 67copper by two ligand-dependent saturable processes. J. Biol. Chem. **263:** 799–805.
71. RUSSANOV, E.M., D.E. DIMITROVA, E.A. IVANCHEVA & M.D. KIRKOVA. 1986. The effects of aspirin, indomethacin and their copper complexes on phospholipase activity and on lipid peroxidation in rat liver microsomes. Acta Physiol. Pharmacol. Bulg. **12:** 36–43.

72. PETERSON, D.A., J.M. GERRARD, G.H. RAO & J.G. WHITE. 1979. Inhibition of ferrous ion induced oxidation of arachidonic acid by indomethacin. Prostaglandins Med. **2:** 97–108.
73. PERRET, D. 1981. The metabolism and pharmacology of *d*-penicillamine in man. J. Rheumatol. (Suppl.) **7:** 41–50.
74. DORR, R.T. 1992. Bleomycin pharmacology: mechanism of action and resistance, and clinical pharmacokinetics. Semin. Oncol. **19**(Suppl. 5)**:** 3–8.
75. SHESKIN, J., R. GORODETZKY, E. LOEWINGER & A. WEINREB. 1981. *In vivo* measurements of iron, copper and zinc in the skin of prurigo nodularis patients treated with thalidomide. Dermatologica **162:** 86–90.
76. WEISMANN, K. 1986. Chelating drugs and zinc. Dan. Med. Bull. **33:** 208–211.
77. PACKER, L., E.H. WITT & H.J. TRITSCHLER. 1995. Alpha-lipoic acid as a biological antioxidant. Free Radical Biol. Med. **19:** 227–250.
78. HURD, R.W., H.A. VAN RINSVELT, B.J. WILDER *et al.* 1984. Selenium, zinc, and copper changes with valproic acid: possible relation to drug side effects. Neurology **34:** 1393–1395.
79. PALM, R. & G. HALLMANS. 1982. Zinc and copper metabolism in phenytoin therapy. Epilepsia **23:** 453–461.